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Short communication

Intravenous anti-influenza drug oseltamivir will not induce torsade de pointes: Evidences from proarrhythmia model and action-potential assay

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ABSTRACT

We evaluated proarrhythmic risk of intravenous oseltamivir with chronic atrioventricular block canine model (n = 4) and action-potential assay on guinea-pig right ventricle (n = 5). Oseltamivir in doses of 3–30 mg/kg, i.v. did not induce torsade de pointes in the canine model, whereas that in concentrations of 30–300 μM decreased maximum rate of phase 0 depolarization, shortened action potential duration at 30%, 60% and 90% repolarization levels, but prolonged difference in action-potential duration between 30% and 90% repolarization levels in a concentration-related manner. These results indicate that oseltamivir will not induce torsade de pointes clinically, since it inhibits both inward and outward currents.

Oseltamivir (Tamiflu®) is a well-established medication against influenza virus infection (1), and its intravenous formulation is being developed for patients who cannot take oral medication because of intubation, unconsciousness and/or vomiting (2,3). Cardiac safety studies using the halothane-anesthetized dogs and isolated guinea-pig atria have shown that intravenous oseltamivir in a dose of 30 mg/kg can suppress the sinus nodal automaticity, ventricular contraction and atrioventricular as well as intraventricular conduction; and delays the ventricular repolarization in a use-dependent manner, which are considered to be exerted through a combination of Na^+, Ca^{2+} and K^+ channel inhibition in the heart (4,5). Meanwhile, phase I studies of single intravenous oseltamivir up to 400 mg have been conducted in healthy subjects (2), and phase II/III studies of intravenous oseltamivir of 100 or 200 mg every 12 h for 5 days for the treatment of influenza were performed in adults and adolescents (3), clarifying that oseltamivir would hardly affect the electrocardiograms, laboratory findings or vital signs (2,3). Since information obtained from healthy subjects or patients with the intact hearts may not necessarily reflect the extent of proarrhythmic risk of intravenous oseltamivir, we evaluated it by using the chronic atrioventricular block canine model (6,7) which is known to have common pathophysiological changes in the heart to those in patients who are the most sensitive to the drug-induced long QT syndrome (8). Furthermore, we assessed the effects of oseltamivir on the action potential of the right ventricular preparation of guinea pigs to confirm its underlying ionic mechanisms (9).

All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No.13-53-152) and the Ethics Committee of Toho University Faculty of Pharmaceutical Sciences (No.05-20), and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University.

A total of 4 beagle dogs of either sex weighing about 10 kg were used. In order to produce proarrhythmia model, the catheter ablation technique of atrioventricular node was employed (6). Briefly, the dogs were anesthetized with thiopental sodium (30 mg/kg, i.v.). After proper positioning of the catheter, the radiofrequency

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energy of 20 W was delivered for 10 s from the tip electrode to an indifferent patch electrode positioned on the animal's back to induce complete atrioventricular block.

Experiments were performed ≥4 weeks after the production of atrioventricular block (6,7). Two hours after the start of the Holter ECG recording (QR2100, Fukuda ME Kogyo, Tokyo), oseltamivir in a dose of 3 mg/kg was intravenously administered over 10 min without anesthesia (n = 4). Holter ECG was recorded for total of 24 h. Then, ≥1 week of washout period after the initial experiments, the effects of 10 mg/kg of oseltamivir were assessed in the same animals (n = 4). Finally, ≥1 week of washout period after the second experiments, the effects of 30 mg/kg of oseltamivir were assessed in the same animals (n = 4). Washout period was determined by the half-life of oseltamivir that was approximately 12 h (10).

A Holter analysis system (HS1000, Fukuda ME Kogyo) was used to examine the electrocardiogram. The QTc was calculated with Fridericia's formula (11). The QT interval, QTc and idioventricular automaticity rate were expressed as the mean of 10 consecutive complexes at pre-drug control (C) and 1, 2, 3, 4, 6, 8, 12 and 21 h after the drug administration. Torsade de pointes was defined as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting ≥6 consecutive beats (12). Also, 51 consecutive beats of electrocardiogram under stable idioventricular automaticity were recorded before and 50–60 min after the start of oseltamivir administration. Poincaré plots with QT_n versus QT_{n+1} were prepared for each of these two analysis time points, of which short-term variability was calculated by the following equation: $(\Sigma|QT_{n+1}-QT_n|/[50 \times \sqrt{2}])$ (7,13).

For the action-potential assay, the hearts were isolated from male Hartley guinea pigs weighing 340–470 g. The right ventricular papillary muscles were quickly dissected and placed in an organ bath containing Krebs–Henseleit solution of the following composition (in mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.1, gassed with 95% O_2/5% CO_2 (pH 7.4 at 37 °C). Cardiac action potential was recorded as previously described (9). Briefly, the preparation was driven at a constant frequency of 1 Hz using an electronic stimulator (SEN-2201, Nihon Kohden, Tokyo) with rectangular current pulses (3-ms duration, about 1.5 times of threshold) through bipolar platinum electrodes. They were impaled with glass microelectrodes filled with 3 M KCl, and transmembrane potential was recorded with a microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo), and analyzed using Chart 7 software (AD Instruments, Dunedin, New Zealand). Action potential parameters measured were resting potential (RP), overshoot (OS), amplitude (AMP), maximum rate of phase 0 depolarization (V_{max}) and action potential duration at 30% (APD_{30}), 60% (APD_{60}) and 90% (APD_{90}) repolarization levels. Difference in action-potential duration between 30% and 90% repolarization levels (APD_{30-90}) was calculated to estimate the extent of I_{Kr}-inhibitory action (14). All experiments were performed at 36.5 ± 0.5 °C.

Oseltamivir was extracted from Tamiflu® Capsule (Chugai Pharmaceutical, Tokyo) with saline in a concentration of 30 mg/mL and its dose was calculated as its free base. The drug was diluted with saline in concentrations of 3 and 10 mg/mL for intravenous use, whereas it was done in distilled water, of which small aliquots provided desired final concentration in the organ bath. The other drugs used were thiopental sodium (Mitsubishi Tanabe Pharma Co., Osaka) and heparin calcium (Sawai Pharmaceutical Co. Ltd., Osaka). The other chemicals were commercial products of the highest available quality.

Data are presented as the mean ± SEM. The statistical significances within a parameter were evaluated by one-way repeated-

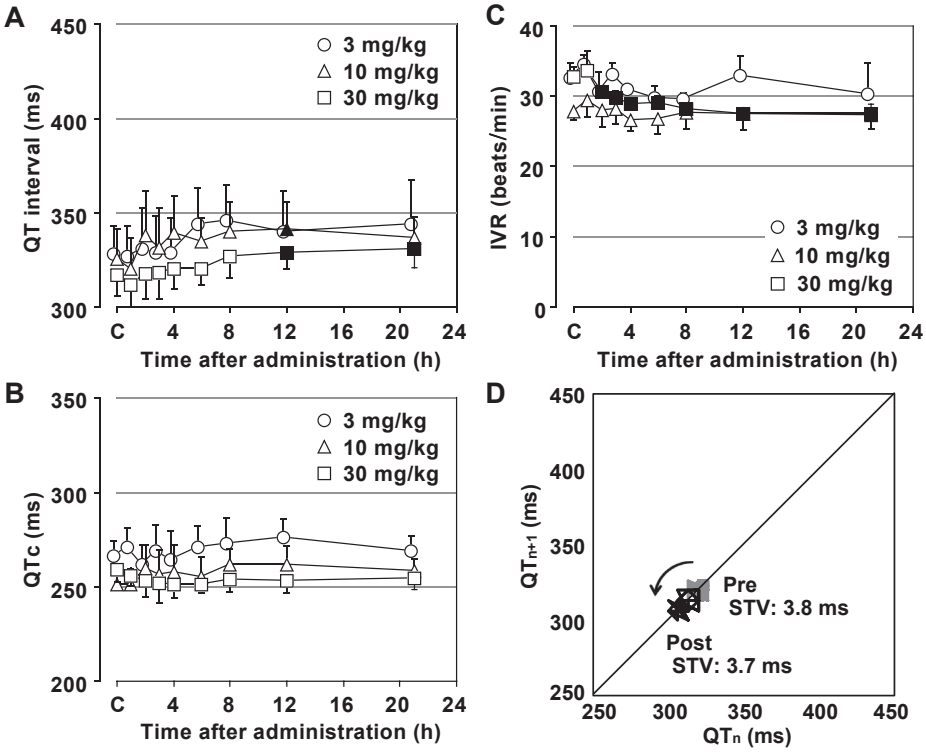

Fig. 1. Summary of electrophysiological effects of intravenous oseltamivir. Time courses of the QT interval (A), corrected QT (QTc) (B) and idioventricular rate (IVR) (C) (n = 4); and typical Poincaré plots showing the beat to beat variability of QT intervals 20 min before and 50 min after the start of administration of 30 mg/kg, i.v. of oseltamivir (D). Oseltamivir neither affected the short-term variability of repolarization (STV) nor induced torsade de pointes. Data are presented as mean ± SEM. Closed symbols represent statistically significant differences from each pre-drug control value (C) by $p < 0.05$.

measures analysis of variance (ANOVA) followed by Contrasts or Dunnet's test as post-hoc test for mean values comparison, whereas those of paired data within a parameter were evaluated by paired *t*-test. A p value < 0.05 was considered to be significant.

The time courses of changes in the QT interval, QTc and idioventricular automaticity rate are summarized in Fig. 1A,B and C, respectively. Oseltamivir in any dose did not induce torsade de pointes, and all animals survived ≥21 h after the administration of each dose. The low dose of 3 mg/kg did not affect the QT interval, QTc or idioventricular automaticity rate. The middle dose of 10 mg/kg prolonged the QT interval at 12 h, but it did not affect the QTc or idioventricular automaticity rate. The high dose of 30 mg/kg prolonged the QT interval for 12–21 h, but it decreased the idioventricular rate for 2–21 h, whereas it did not affect the QTc. Typical Poincaré plot of QT intervals for the high dose is depicted in Fig. 1D. The short-term variability of the QT interval before and after the drug administrations (n = 4 for each dose) was 3.3 ± 0.6 and 3.4 ± 0.6 ms for the low dose, 3.9 ± 0.6 and 3.6 ± 0.6 ms for the middle dose, and 3.9 ± 0.6 and 2.9 ± 0.6 ms for the high dose, respectively. Oseltamivir tended to shorten the short-term variability in a dose-related manner, although it did not achieve statistical significance.

Typical tracings showing the effects of oseltamivir on the action potential waveform are depicted in Fig. 2, and the summary of its effects on the action-potential parameters is shown in Table 1. Oseltamivir in concentrations of 30–300 μM decreased the OS, AMP and V_{max}, shortened the APD_{30}, APD_{60} and APD_{90}, but prolonged the APD_{30-90} in a concentration-related manner.

Intravenous oseltamivir did not induce torsade de pointes in the chronic atrioventricular block dogs, but tended to shorten its short-term variability, rather reflecting modest antiarrhythmic property. Oseltamivir in intravenous doses of 100, 200 and 400 mg over 2 h in phase I study (2) provided C_{max} of 0.263, 0.548 and 1.100 μg/mL, respectively, and in our previous study using the halothane-anesthetized dogs, oseltamivir in intravenous doses of 0.3, 3 and 30 mg/kg over 10 min provided peak plasma concentration of 1.2, 10.6 and 117.5 μg/mL, respectively (5), suggesting that plasma concentration in this study would be about 10–100 times higher than its therapeutic one. To better clarify its underlying ionic mechanism, we assessed the effects on the action potential waveform. Oseltamivir decreased the V_{max}, APD_{30} and APD_{60}. The former indicates Na^+ channel blocking property, whereas the latters suggest Ca^{2+} and late Na^+ channel inhibition (15). Moreover, the APD_{30-90} was prolonged by oseltamivir, suggesting I_{Kr} channel inhibitory action (14). It should be noted that the APD_{90} was shortened by oseltamivir, indicating that inhibition of inward currents may be greater than that of outward ones. These in vitro observations may partly explain why oseltamivir suppressed the idioventricular automaticity but did not induce torsade de pointes in vivo. Oseltamivir carboxylate, a major active metabolite of oseltamivir did not inhibit I_{Kr} channel according to the information described in the interview form from the manufacturer; however, further study is needed to confirm the metabolites of oseltamivir in dogs are identical to those in human. In conclusion, these in vivo and in vitro observations may suggest that intravenous oseltamivir by itself has no potential risk to induce torsade de pointes.

Table 1
Effects of oseltamivir on myocardial action potential parameters of the guinea-pig right ventricular preparation.

		Control	Oseltamivir 30 μM	Oseltamivir 100 μM	Oseltamivir 300 μM
RP	mV	−84.3 ± 0.7	−83.5 ± 0.5	−82.8 ± 0.5	−81.7 ± 0.5
OS	mV	37.9 ± 1.3	37.4 ± 1.8	35.1 ± 2.0	31.9 ± 2.6*
AMP	mV	122.2 ± 1.3	121.0 ± 1.8	117.9 ± 2.4	113.6 ± 3.0*
V_{max}	V/s	209.9 ± 8.6	209.1 ± 7.0	183.8 ± 4.5*	151.9 ± 6.9***
APD_{30}	ms	152.9 ± 7.4	149.9 ± 8.5	142.1 ± 8.7**	126.2 ± 9.9**
APD_{60}	ms	190.7 ± 5.4	188.9 ± 6.8	184.9 ± 6.7	171.1 ± 7.1**
APD_{90}	ms	205.1 ± 5.9	203.5 ± 6.2	200.7 ± 6.0	188.5 ± 6.4*
APD_{30-90}	ms	52.1 ± 3.5	53.6 ± 3.1	58.6 ± 3.5	62.3 ± 4.2*

Data are expressed as mean ± SEM of 5 experiments. Effects of oseltamivir were assessed 15 min after the drug application. Action potential duration at 30% (APD_{30}), 60% (APD_{60}) and 90% (APD_{90}) repolarization, resting potential (RP), overshoot (OS), amplitude (AMP) and maximum rate of phase 0 depolarization (V_{max}). The triangulation parameter (APD_{30-90}) was defined as the difference between APD_{90} and APD_{30}. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control.

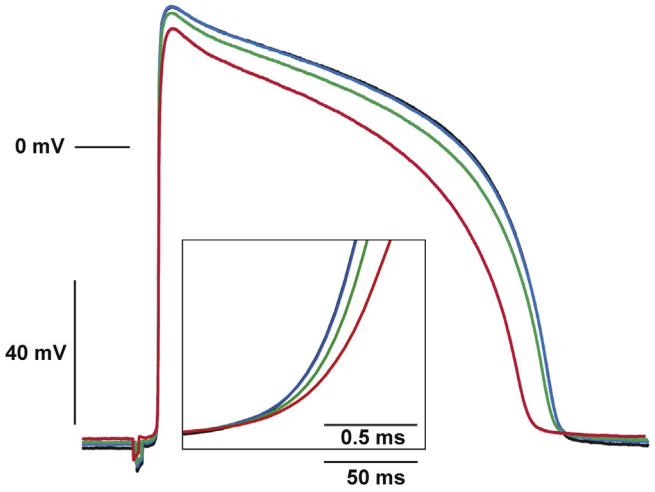

Fig. 2. Typical tracings showing the effects of oseltamivir on the action-potential waveform of the right ventricular preparation of guinea pigs. The preparation was electrically driven at 1 Hz. Black, blue, green and red lines indicate control, 30 μM, 100 μM and 300 μM of oseltamivir, respectively. Black and blue lines run on almost identical trace, suggesting that oseltamivir of 30 μM did not affect the action potential waveform. Inserted figure shows the magnification of phase 0 depolarization, in which the horizontal scale is 100 times greater than that of original one, whereas the vertical scale is common to both figures.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

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